

THE LIFE HISTORY, DEVELOPMENTAL STAGES, AND TAXONOMIC  
RELATIONS OF THE DIGENETIC TREMATODE *LASIOLOCUS*  
*MINUTUS* (MANTER, 1931) THOMAS, 1959<sup>1</sup>

HORACE W. STUNKARD

*The American Museum of Natural History, Central Park West at 79th Street, New York,  
N. Y. 10024; and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543*

ABSTRACT

*Cercaria adranocerca* n. sp. was described by Stunkard and Uzmann (1959) from the clam *Gemma gemma* at Boothbay Harbor, Maine. Further study of the species at Woods Hole, Massachusetts, has afforded information for revision and correction of the original description. The cercariae are microcercous and after emergence from their sporocysts are encysted in the haemocoel of the clam. They may be extruded, either singly or embedded in a jelly-like matrix, and float in seawater. Feeding metacercariae to *Menidia menidia* yielded developmental stages to mature worms, identified as *Lasiotocus minutus* (Manter, 1931) Thomas, 1959. Discovery of the life cycle may aid in resolution of the controversy concerning the status of the genera *Lasiotocus* Looss, 1907; *Genolopa* Linton, 1910; and *Proctotrema* Odhner, 1911.

INTRODUCTION

*Lasiotocus minutus*, a parasite in the intestine of *Menidia menidia*, was described and named by Manter (1931) from specimens taken at Beaufort, North Carolina. It is a member of the Monorchhiidae, a large family with 12 subfamilies, about 30 genera, and 100 species, which infect marine fishes in all parts of the world. Observations on different stages in the life history of certain species have been recorded and the complete life cycle of a single species, *Monorcheides cumingiae*, was worked out and published by Martin (1940).

In a paper on the life history of *Proctoeces maculatus*, Stunkard and Uzmann (1959) included the description of a microcercous cercaria produced in sporocysts in the small bivalve *Gemma gemma*. The clams were taken in August and September, 1957, in the region of Boothbay Harbor, Maine. The asexual generations were described under the designation, *Cercaria adranocerca*, and type specimens were deposited in the U. S. National Museum under the number 56236. Although the excretory vesicle was saccate rather than Y-shaped, the cercariae were microcercous and otherwise similar to those of *Proctoeces maculatus*; accordingly, they were assigned tentatively to the family Fellodistomidae. The species was found in *G. gemma* of the Woods Hole region and further study has shown it to be the larval stage of *Lasiotocus minutus*.

MATERIAL AND METHODS

Between 1 June and 1 August 1980 more than 4000 specimens of *G. gemma* were collected from different locations in the area of Woods Hole. Infection with *C. adranocerca* varied from 2 to 5%. Most of the clams were maintained in small

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bowls, 25 to 50 in each bowl, to obtain metacercariae. With a set of 25 bowls, metacercariae were available almost every day. Completion of the life cycle proved *Cercaria adranocerca* to be the larval stage of *Lasiotocus minutus*.

Dissection of clams provided the asexual generations of the species. There are at least two generations of sporocysts (Figs. 1, 2) with individuals of varying sizes in the haemal sinuses of *G. gemma*. They invade both the digestive gland and the gonad. Small individuals contain a few germ-balls and incipient cercariae while large ones, filled with developing cercariae, reach 1.20 mm in length and 0.20 mm in diameter. The birth pore is terminal and this end may be protruded. Small specimens are motile, but large, gravid sporocysts are incapable of movement.

The cercariae are microcercous, without stylets or ocelli, and are not liberated from the mollusk. Rather, they are encysted while in the haemocoel of the clam. Here, they may aggregate in clusters (Fig. 3), or may be extruded either singly or in strands (Fig. 4) embedded in a jelly-like matrix. When liberated in the sea, the metacercariae, either individually or in clusters, float and are carried about by water currents. Eventually they sink to the bottom and may be ingested by small crustaceans or other carnivorous invertebrates. When discharged in strands, each mass may contain as many as 500 metacercariae (Fig. 4). The emergence of metacercariae apparently is not determined by either light or temperature. They are found early in the morning and late in the afternoon. Floating strands of metacercariae, often embedded in bits of clam or other material, were fed to small fishes: *Menidia menidia*, *Fundulus heteroclitus*, and flatfishes (0-year *Pseudopleuronectes americanus*). The fishes were collected in adjacent waters and were naturally infected. At Woods Hole, *M. menidia* harbors two species of *Lasiotocus*: *L. minutus* (Manter, 1931) Thomas, 1959 and *L. elongatus* (Manter, 1931) Thomas, 1959 (described in Stunkard, 1981). Developmental stages of the two species may overlap in size but may be distinguished by specific characters and especially by the sizes of suckers. In *L. minutus*, the oral sucker is slightly larger than the acetabulum, whereas in *L. elongatus* the acetabulum is the larger. For experimental infections, the fishes were isolated for about 2 weeks and fed on living tissues that did not contain parasites. Then, at intervals of about 1 week, they were fed metacercariae shed by *G. gemma*. Large strands were added to the aquaria and pieces were embedded in bits of clam or other tissue that insured that they would be eaten. Dissection of fishes at intervals yielded all stages of development of *L. minutus*, from metacercariae to gravid adults, and established that *C. adranocerca* is the larva of that species. The infection of fishes occurs without the intervention of a second intermediate host, but the metacercariae may be eaten by small crustaceans and their ingestion by *M. menidia* is not precluded. No infection was established in either *F. heteroclitus* or *P. americanus*.

#### DESCRIPTIONS

##### *Sporocysts*

Stunkard and Uzmann (1959) reported that in *C. adranocerca* the sporocysts (Figs. 1, 2) were relatively few and oval to sausage-shaped; the largest 0.42 mm long and 0.11 mm wide; and smaller ones, no larger than a cercaria, containing a few germ balls. Study of more abundant material permitted additions to the description. The number of sporocysts was small, but gravid specimens, more than 1 mm long and 0.13 mm wide, were commonly present and filled with developing cercariae. Since there are successive generations of sporocysts, it is apparent that the cercariae are produced in daughter sporocysts. The daughters are recognizable

when very small and may be distinguished from cercariae by the formation of an internal lumen, containing a few morula-like clusters of more deeply staining cells. As development proceeds, it appears that the progeny consists of a few daughters and then the sporocysts produce only cercariae.

### *Cercariae*

A representative account of the cercaria (Figs. 2, 7) was given by Stunkard and Uzmann (1959). The data were taken from specimens found on dissection of two clams and it is now evident that those infections were recent, not fully mature. The measurements were smaller than those obtained in the study of specimens in older and well established infections. The earlier account reported cephalic-gland ducts in the region of the oral sucker. That observation has not been confirmed. From the level of the pharynx to the posterior ends of the caeca, cystogenous glands underlie the body wall, except for the areas occupied by the excretory vesicle and reproductive organs. Extrusion of secretion from these glands in the oral area probably was misconstrued in the earlier report. Since the cercariae do not invade a second intermediate host, there is no reason for penetration glands.

Heat-fixed cercariae had the following average measurements: length, 0.17 mm; width, 0.052 mm; acetabulum, 0.025 mm; oral sucker, 0.030 mm; pharynx, 0.014 mm. The tail, 0.006 mm in diameter, is attached ventrally. There are two lateral excretory pores at the body-tail junction. The excretory vesicle was described as saccate, but as it is filled with fluid and spherical concretions, it may extend forward to the acetabulum. In this condition (*i.e.*, when extended) the collecting ducts open into the vesicle some distance posterior to its anterior end.

The 1959 report tentatively assigned *Cercaria adranocerca* to the family Felodistomidae. Further information has demonstrated that the species is a member of the family Monorchiidae. The earlier error is explained by the report of Martin (1940) that in *Monorcheides cumingiae*, the only marine species whose life cycle was then known, the cercaria is biocellate and has a long tail with lateral cup-shaped lappets. However, Cable (1956) reported monorchiid cercariae from Puerto Rico, one species with eye-spots and a long tail like *M. cumingiae* and another without ocelli and with the tail reduced to a tiny knob. His (1965) review of the subject of cercarial tails submitted evidence that tails may be deceptive and misleading. The same type may be present in different families and different types in the same family.

### *Metacercariae*

Existence as cercariae is brief and transitory. Soon after it emerges from the sporocyst, each cercaria begins a series of writhing movements that cause the emission of the secretion from the cystogenous glands. This colorless, transparent material forms a large, loose, flexible, membranous sac around the metacercaria (Figs. 3, 4). The distal tip of the sac, especially, is adhesive. When infection is heavy the metacercariae may form clusters (Fig. 4). When the number of the cercariae is small, they may be extruded singly, or two or more may attach to a bit of debris or to each other (Fig. 8). In such cases, on fixation, the walls of the sacs collapse, forming filaments 0.15 to 0.18 mm long, connecting the metacercariae to the object or to each other. The metacercariae, fixed, stained and mounted, measure 0.07–0.08 mm in diameter. Floating strands of living metacercariae (Fig. 9) may measure 5.00 mm in length and 0.90 mm in width. It appears that the size and shape of the strand is determined by the diameter of the siphon.

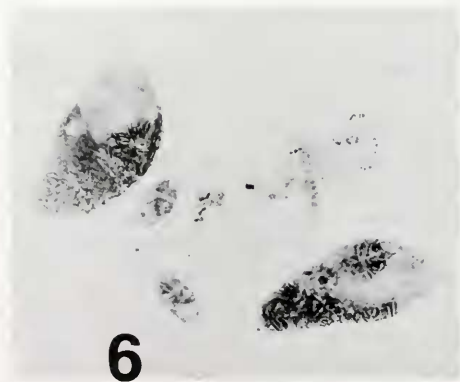
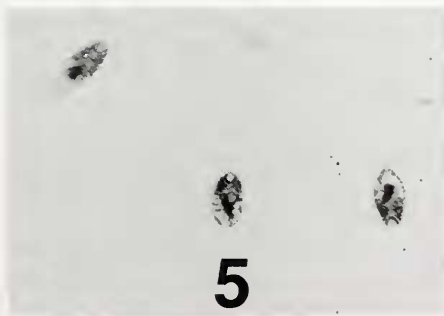
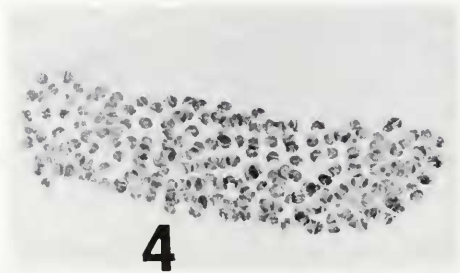
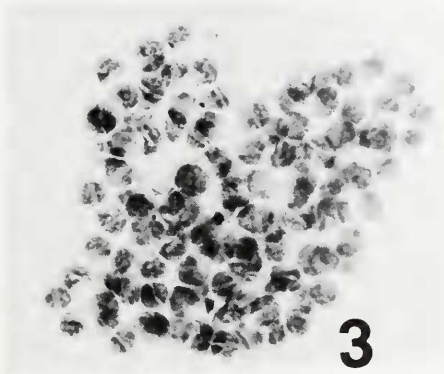
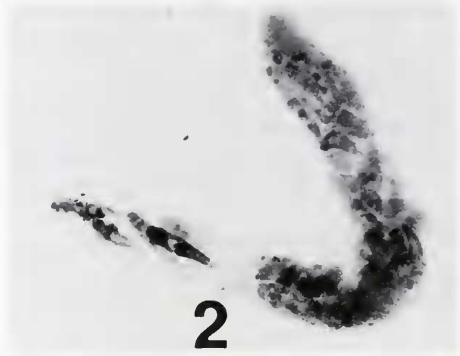
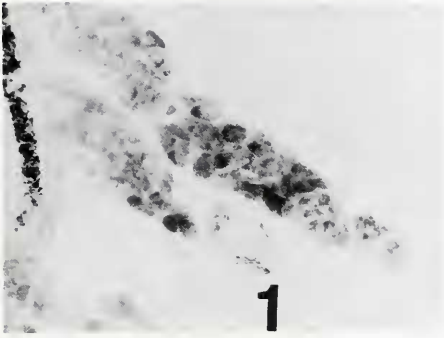


FIGURE 1. Ends of sporocysts in tissue of *G. gemma*, 0.16 mm in diameter. The black material is in the rectum of the clam.

FIGURE 2. Isolate sporocyst with germ-balls and cercariae; anterior and extended. Total length 1.16 mm.

FIGURE 3. A cluster of encysted cercariae in the mantle cavity of the clam, each 0.045 mm in diameter, alive, and active.

FIGURE 4. A strand of metacercariae after extrusion from *G. gemma*, embedded in matrix, floating in bowl, alive;  $5.7 \times 0.94$  mm.

FIGURE 5. Juvenile specimens, 10 days development in *M. menidia*, fixed, stained and mounted; specimens  $0.115 \times 0.046$  mm.

FIGURE 6. Mature and juvenile specimens, natural and experimental infections in the same fish, fixed, stained and mounted; mature specimens,  $0.325 \times 0.130$  mm; juveniles,  $0.09 \times 0.05$  mm.

*Adults*

The adult worms (Figs. 11, 12) recovered from *M. menidia* were identified as *Lasiotocus minutus*. Gravid specimens measured 0.30–0.70 mm in length and 0.10–0.25 mm in width. They are almost cylindrical and taper toward both ends, although more rounded anteriorly. The integument bears closely set, flattened spines disposed in quincuncial arrangement. They diminish in size and number posteriorly. The body wall is composed of the usual circular, longitudinal and diagonal muscular layers; it is strong and well developed for such a small worm. The acetabulum shifts forward as the reproductive organs mature, from near midbody to the anterior third. It is somewhat smaller than the oral sucker, 0.042–0.050 mm in diameter. Both suckers appear larger when the lumen is expanded and the opening is enlarged. The oral sucker is subterminal, 0.052–0.058 mm in diameter, and the mouth is ventral. The short prepharynx and somewhat longer esophagus may both disappear when the anterior end of the worm is retracted. The pharynx is 0.020–0.025 mm in diameter; the digestive tract bifurcates a short distance anterior to the acetabulum and the caeca terminate near the anterior end of the excretory vesicle when it is saccate and relatively empty. The genital pore is located immediately anterior and somewhat lateral to the acetabulum and opens from a common genital atrium. The single testis, typically oval, 0.05–0.07 mm in diameter, is located near midbody but shifts anteriorly as the posttesticular region fills with uterine coils. The cirrus sac is clavate; it extends over and posterior to the acetabulum and under coverglass pressure may be displaced to the right or left. The sac contains a large, oval seminal vesicle followed by the prostate and the ejaculatory duct, which may be protruded as a cirrus. The duct is lined with small triangular spines 0.004–0.005 mm in length with curved tips. The ovary is spherical to oval, 0.025–0.030 mm in diameter, situated anterior or ventral to the anterior part of the testis. The oviduct arises from the posterior end and is joined by the duct from the seminal receptacle, which continues, as Laurer's canal, to open on the dorsal side of the body. The oviduct then receives the common vitelline duct and enters Mehlis' gland to become the ootype. It is continued by the initial part of the uterus. The proximal part of the uterus contains spermatozoa and continues as a much coiled series of loops that fill the posterior half of the body, covering the gonads and the lateral areas of the body to the level of the pharynx. The metraterm is short, bearing conical spines 0.005–0.006 mm in length with rounded bases. It enters the genital atrium ventral and posterior to the opening of the ejaculatory duct. Eggs in the initial portion of the uterus have thin, transparent shells, and the embryos stain intensely; eggs in the distal coils have thick yellow shells and measure 0.020–0.022 mm in length and 0.012–0.013 mm in width. The eggs are operculate, with an opening 0.008–0.009 mm in diameter. They are embryonated when passed, but the miracidia do not emerge when maintained in seawater. The egg shells are firm, but under coverglass pressure they may flatten and appear slightly larger. The vitellaria consist of dorsal follicles, and lateral to the ovary. Vitelline ducts pass mediad on each side and unite to form a vitelline receptacle that discharges into the oviduct just before it enters Mehlis' gland. A few vitelline cells and a single ovum are incorporated into each egg in the gland. The description of the excretory system, vesicle, tubules, and flame-cells is in the original account (Stunkard and Uzman, 1959). The vesicle was described as saccate, but it may extend forward toward the acetabulum as it fills with fluid and spherical concretions. In this condition, the collecting ducts open into the vesicle some distance from its anterior end.

The worms agree substantially with the diagnosis of the species as given by Manter (1931), but not with his figure 2 of the species, in which the ovary and vitellaria are some distance posterior to the acetabulum. Also, it is obvious that the egg size given by Manter is for collapsed, not normal, eggs. Manter (1931) listed *Monostomum* sp. Linton 1905, p. 356, from *Menidia menidia* and *Fundulus majalis* at Beaufort, North Carolina as a synonym of *Genolopa minuta*. Actually, that species is a synonym of *Lasiotocus elongatus*, not *Lasiotocus minutus*.

#### DISCUSSION

The integrity and validity of the genera *Lasiotocus* Looss, 1907; *Genolopa* Linton, 1910; and *Proctotrema* Odhner, 1911, have long been in dispute. *Lasiotocus* was based on *Distoma mulli* (Stossich, 1883) from *Mullus barbatus* taken at Trieste in the Adriatic. *Genolopa* was based on *Genolopa ampullacea* n. sp., from *Haemulon* spp., and other fishes at Tortugas, Florida, and included other specimens described and figured as *Monostomum* sp. in Linton (1907), from the same host-fishes taken in Bermuda. *Proctotrema* was based on *Proctotrema bacilliovatum* n. sp., from *Mullus barbatus* taken at Trieste. The worms were from the same host-species and location as *Lasiotocus mulli* (Stossich, 1883) and their relations were considered. Odhner (1911, p. 250) stated, "Hieraus geht indessen hervor, dass sich diese Form unter dem vom Verfasser eingesammelten Triester Materiale befindet und von ihm schon untersucht worden ist; unter solchen Umständen möchte ich den Mitteilungen des älteren Kollegen nicht vorausgreifen, sondern begnüge mich damit festzustellen dass *Lasiotrema mulli* mit *Proctotrema bacilliovatum* nächstverwandt ist." He described the eggs as 31–33 by 8–9  $\mu\text{m}$  and reported, p. 250, "sie legen sich mit parallel Langsachsen in kleinen Bündeln zusammen." This statement is a misrepresentation, from collapsed, distorted eggs. Linton's paper was not available to Odhner.

Synonymy of *Genolopa* and *Proctotrema* has been discussed by many authors. Lloyd and Guberlet (1932) declared that information was too inadequate to warrant a decision. Yamaguti (1934) predicated the probable identity of *Genolopa* and *Proctotrema*, since it was apparent that Linton (1910) had overlooked the acetabulum in *Genolopa*. Indeed, Linton did not mention the acetabulum; he described a genital sucker, actually the genital atrium, located immediately anterior to the acetabulum. Manter (1940) distinguished between *Genolopa* and *Proctotrema* by differences in spination of the copulatory organs. He suggested that *Genolopa* be restricted to species with a median cluster of longer spines in the cirrus sac, a feature of *G. ampullacea*. Referring to *Genolopa*, Hopkins (1941) declared, p. 399, "the type specimen agrees with Odhner's (1911) description of the genus *Proctotrema* in every point except for the more rounded shape of the oral sucker, the non-lobate form of the ovary (which may be a result of flattening, since the ovary in other species with tri-lobate ovary is more rounded in flattened specimens), and the less elongate shape of the eggs in *G. ampullacea*. Since these are minor differences, and since other species completely cover the range of differences in even these characteristics, it is obvious that *Proctotrema* Odhner, 1911, is a synonym of *Genolopa* Linton, 1910. In spite of Manter's statement, the spination of the cirrus in *G. ampullacea* is in no way exceptional, and there is no 'median cluster of much longer spines in the cirrus sac' in the type specimen." Hopkins transferred all described species from *Proctotrema* to *Genolopa*. Manter (1942) described nine species of monorchiid trematodes from fishes at Tortugas, Florida. He gave a

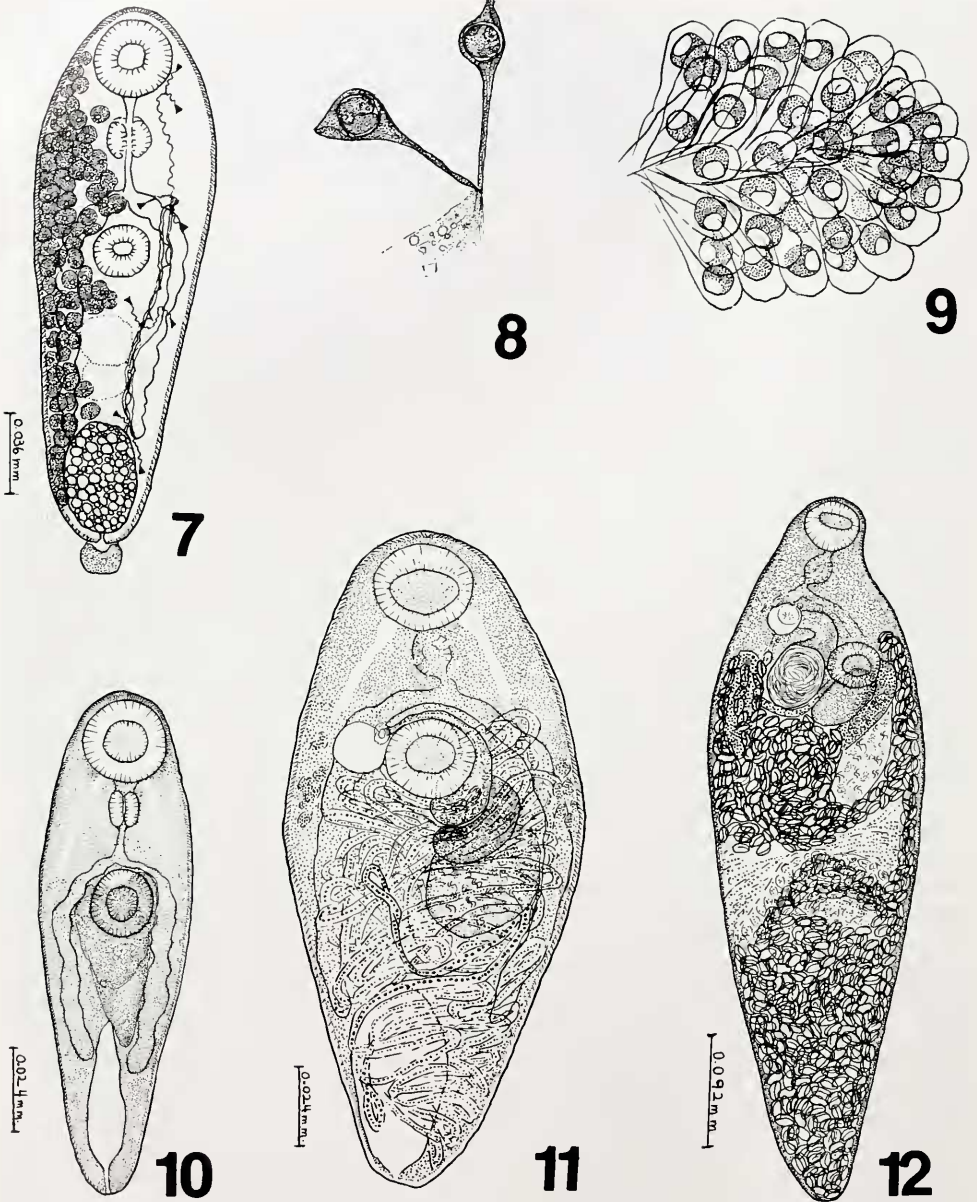


FIGURE 7. Cercaria, drawn from pencil sketches; cystogenous gland cells on one side, excretory system on the other side.

FIGURE 8. Isolated metacercariae attached by filaments to a bit of debris; fixed, stained and mounted.

FIGURE 9. End of a strand of floating metacercariae embedded in matrix, drawn from a living specimen.

FIGURE 10. Juvenile specimen from *M. menidia*, experimental infection; fixed, stained and mounted, 0.15 mm long.

FIGURE 11. Mature but not fully gravid specimen (no shelled eggs) fixed, stained and mounted; length 0.196 mm. Drawn to the same scale as Figure 10.

FIGURE 12. Fully gravid specimen, fixed, stained and mounted, length, 0.68 mm.

detailed description of *G. ampullacea*, formulated a new diagnosis of *Genolopa*, and insisted on the validity of *Proctotrema*. Manter and Pritchard (1961) reported on digenetic trematodes of Hawaiian fishes. They accepted the validity of *Lasiotocus*, *Genolopa*, and *Proctotrema*, but added, p. 483, "The taxonomy of the family Monorchiidae is in a state of considerable uncertainty."

The status of *Lasiotocus* remains equivocal. Dollfus (1948) gave a detailed description of *L. mulli*, with figures of an entire specimen and of the copulatory organs. Thomas (1959) described five species of monorchiid species from fishes taken on the coast of Ghana. He reviewed the family Monorchiidae, gave a revised diagnosis of *Lasiotocus*, and suppressed *Proctotrema* as a synonym of *Lasiotocus*. Accordingly, he transferred some 16 species, including *Genolopa minuta* Manter, 1931, to *Lasiotocus*. Bartoli and Prévot (1966) gave a new description of *Proctotrema bacilliovatum* Odhner, 1911, the first study of the species since Odhner's 1911 original description. The specimens came from the rectum of *Mullus barbatus* at Trieste. The description by Odhner was brief and inadequate; the account by Bartoli and Prévot detailed and well illustrated. They reported that *P. bacilliovatum* appeared to be distinctive, but all species previously assigned to *Proctotrema*, except *P. bacilliovatum*, were transferred to *Lasiotocus*.

In his *Synopsis of digenetic trematodes of vertebrates*, Yamaguti (1971) listed 12 subfamilies in the family Monorchiidae but the family diagnosis was so diverse that its unity was impaired. The subfamily Lasiotocinae contained 20 genera, including *Lasiotocus*, *Genolopa* and *Proctotrema*. Data for the precise systematic determination of these trematodes is still inadequate. Knowledge of their asexual generations and intermediate hosts may help resolve the situation.

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